Two affected siblings with nuclear cataract associated with a novel missense mutation in the CRYGD gene

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Purpose: To identify the disease locus for nuclear congenital cataract in a nonconsanguineous family with two affected members.

Methods: One family with two affected members with congenital cataract and 170 normal controls were examined. DNA from leukocytes and bucal swabs was isolated to analyze the CRYGA-D cluster genes and microsatellite markers D2S325, D2S2382, and D2S126, and to discard paternity through gene scan with several highly polymorphic markers.

Results: DNA sequencing analysis of the CRYGA-D cluster genes of the two affected members showed a novel heterozygous missense mutation c.320A>C within exon 3 of the CRYGD gene. This transversion mutation resulted in the substitution of glutamic acid 107 by an alanine (E107A). Analysis of the two unaffected members of the family and the normal parents showed a normal sequence of the CRYGA-D cluster genes. This mutation was not found in a group of 170 unrelated controls. We consider that it is unlikely that this abnormal allele represents a rare polymorphism. DNA analysis showed no evidence for non-paternity while genotyping indicated that the haplotype of the mother co-segregated with the disease.

Conclusions: In this study we describe the mutation c.320A>C (E107A) in the CRYGD gene associated with nuclear congenital cataract. Haplotype analysis strongly suggests that the origin of the mutation was transmitted through the mother.

Cataract is the leading cause of reversible blindness in childhood with an occurrence of 1-6/10,000 live births [1-3]. It is due to different causes including metabolic disorders, infections during embryogenesis, gene defects and chromosomal abnormalities [2]. In general, cataract is the consequence of breakdown of the lens microarchitecture that produces changes in refraction index and light scattering. Inherited cataracts correspond between 8 and 25% of congenital cataracts [4,5]. They are characterized by the location and structure of opacities (i.e., shape, size, color, and refractive quality). Congenital or infantile cataract is visible within first year of life while juvenile cataract is present within the first decade. Age-related cataract occurs after the age of 45 years. However, the age of onset is not necessarily related with the etiology of cataract. More than one classification system of human inherited cataracts has been developed based on the anatomic location (i.e., nucleus, posterior capsule, cortex) or morphology of the opacity (i.e., lamellar, sutural, pulverulent), nevertheless, classification has been difficult due to wide phenotypic variability [6-10].

Cataract may be an isolated anomaly or part of a syndrome. The majority of inherited non-syndromic cataracts are transmitted as an autosomal dominant trait but X-linked and autosomal recessive inheritances have also been reported [4].

Inherited cataracts are clinically heterogeneous with inter and intrafamilial variability [11]. Congenital cataracts are also genetically heterogeneous. Phenotypically identical cataracts are the result of the molecular defect at different loci and phenotypically variable cataracts have been observed with the same gene defect [3,12-14]. Linkage analysis is a useful tool to identify different loci that can result in human cataract. From the first description of the cosegregation of inherited cataract with the Duffy blood group locus [15] more than 20 loci have been linked with congenital cataracts [10,16]. Mutations in the CRYG genes, which encode the main cytoplasmic proteins of the human lens, have been associated with cataracts of various appearances. Crystallin proteins (α-, β-, and γ-crystallin) represent more than 90% of the soluble proteins of the lens in human. Whereas α-crystallins are heat shock proteins, β-crystallins and γ-crystallins are included in a superfamily of microbial stress proteins.

Nuclear congenital cataract is uncommon and suggests an anomaly in early development. In general, opacities are confluent and discrete, with bilateral symmetrical involvement. An exception is pulverulent cataract in which the type of distribution of nuclear opacities can vary between affected siblings and in the same patient [1,11]. Several genes have been associated with the presence of nuclear cataract (i.e., CRYBA3/A1, CRYGD, CRYGS, GJA3, and GJA8) [17-23]. Genes ranging from CRYBA3 to CRYGS seem to be the most frequently involved in the pathogenesis of nuclear cataract with sutural components. Since the number of mutations in the CRYGA-D gene in dominant cataracts is high in humans, this gene clus-
ter was analyzed as a candidate. In the present study we analyzed two affected members, products of normal and non-consanguineous parents, in a family with nuclear congenital cataract and identified a novel missense mutation within exon 3 of the CRYGD gene.

**METHODS**

A family was referred to the Ophthalmology Department by the presence of congenital cataract. Protocol was approved by the Ethics Committee of the General Hospital of México. All participants gave informed consent to the study. The family included 2 affected patients and 2 unaffected subjects, products of normal and non-consanguineous parents (Figure 1).

To perform molecular analysis of the CRYGA-D cluster, we obtained genomic DNA from peripheral blood and bucal swab with conventional methods. Conditions to amplify exons through PCR were as follows: DNA 500 ng, primers 0.4 µM, dNTP's 0.08 mM, MgCl2 1.5 mM, buffer 1X, Taq Pol 1.5 U, in a total volume of 50 µl at 94 °C for 1 min, 30 cycles of 94 °C for 1 min, and 72 °C for 2 min. Exon primers and annealing temperature are described elsewhere [18,22,23]. PCR products were purified with a PCR purification kit (Qiaex II, Qiagen, Hilden, Germany). DNA sequence analysis was performed using an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer’s conditions. To identify paternity we genotyped DNA from peripheral blood and bucal swabs with several highly polymorphic markers (Table 1). This segregation analysis was performed through GeneScan using the ABI Prism Linkage Mapping Set (Applied Biosystems). In an attempt to identify which parent haplotype co-segregated with the disease we haplotyped the pedigree with microsatellite markers D2S325, D2S382, and D2S126. All assays were performed two times with a normal control included. Clinical characteristics of lens opacities were analyzed by slit-lamp. The method for assessing length was the “A scan technique” with an OcuScan Biophysic Alcon Biometer (version 3.02). SRK-T formula was used to calculate intra ocular lens power.

**Table 1.**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allelic frequency in Hispanic populations</th>
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<tbody>
<tr>
<td>CSF1PO</td>
<td>0.0465</td>
</tr>
<tr>
<td>TPOX</td>
<td>0.0023</td>
</tr>
<tr>
<td>THO1</td>
<td>0.0365</td>
</tr>
<tr>
<td>FESFPS</td>
<td>0.0789</td>
</tr>
<tr>
<td>F13A01</td>
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<tr>
<td>VWA</td>
<td>0.0052</td>
</tr>
<tr>
<td>D16S539</td>
<td>0.0620</td>
</tr>
<tr>
<td>D7S820</td>
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</tr>
<tr>
<td>D13S317</td>
<td>0.0028</td>
</tr>
<tr>
<td>FGA</td>
<td>0.0050</td>
</tr>
<tr>
<td>D8S91179</td>
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</tr>
<tr>
<td>D18S551</td>
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<tr>
<td>D3S1744</td>
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</tr>
<tr>
<td>F13B</td>
<td>0.1100</td>
</tr>
<tr>
<td>HPRTB</td>
<td>0.0320</td>
</tr>
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</table>

Highly polymorphic markers used in the test of paternity. Probability of random coincidence is 1.01x10^{-11}. All markers were identical in the patients and their father.

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Figure 1. Pedigree of the family. Subjects II:1 and II:4 were the affected patients with the c.320A>C mutation in the CRYGD gene. Parents I:1-I:2 and sibs II:2-II:3 with no cataract showed no mutations. Segregation of microsatellite marker D2S325 is also shown.
RESULTS
The proband (II:1; Figure 1) was a 35-year-old male. He was the product of young and nonconsanguineous parents. Onset of symptoms was at age of 4 years with diminution of visual acuity (VA), nystagmus, and photophobia. A diagnosis of nuclear cataract was made. Upon clinical examination, the patient achieved VA of 20/400 in both eyes. The reduction in vision was long standing and progressive. The characteristics of the cataract is shown in Figure 2A. He presented myopia of high degree (RE 28.32 mm and LE 26.76 mm of antero-posterior diameters). The cycloplegic refraction showed: RE -9 spherical equivalent and LE -7.50 spherical equivalent. No other ocular findings were found to be present.

The sister (II:4; Figure 1) of the proband was a 30-year-old female. Onset of symptoms was at age of 5 years with diminution of VA and photophobia. A diagnosis of nuclear cataract was made. On clinical examination the patient achieved a VA of 20/400 in both eyes. The reduction in vision was long standing and progressive. Bilateral cataract is shown in Figure 2B. She presented myopia with the followings antero-posterior diameters: RE 25.11 mm, LE 25.69 mm. The cycloplegic refraction showed: RE: -3.50 spherical equivalent and LE: -2 spherical equivalent. No other ocular findings were found to be present.

DNA sequencing analysis of the CRYGA-D cluster genes of the two affected members of the family showed a novel heterozygous missense mutation c.320A>C within exon 3 of the CRYGD gene (Figure 3). This transversion mutation resulted in the substitution of glutamic acid 107 by an alanine (E107A). The A>C transversion did not result in the gain or loss of a convenient restriction site. Analysis of the two unaffected sibs and their normal parents showed a normal sequence of the CRYGA-D cluster genes. The missense mutation c.320A>C co-segregated in the affected members of the family and was not found in a group of 170 unrelated controls. We therefore consider that it is less likely that this abnormal allele represents a rare polymorphism. Particularly, after sequencing the coding region of CRYGD of both parents with no cata-
ract and no molecular defects, we genotyped DNA (peripheral blood and bucal swab) and found no evidence for non-paternity. Haplotyping of the pedigree, especially microsatellite marker D2S325, indicated that the mother’s haplotype co-segregated with the disease (Figure 1); microsatellite markers D2S2382 and D2S126 were not informative.

**DISCUSSION**

Crystallins are critical to lens function and play a structural role for transparency and refraction [24]. The soluble fractions of lens proteins comprise the α-, β-, and γ-crystallins. In the human lens, γ-crystallins represent 25% of the total crystallin proteins. They are subdivided into two groups: γABC- and γDEF-crystallins [25,26], in human γE- and γF-crystallin are pseudogenes and are not expressed in other tissues [17]. γC- and γD-crystallin are the principal human γ-crystallins. The genes that encode γ-crystallin encompass 3 exons, the first exon encodes 3 amino acids, the second exon encodes the first and second Greek key motifs and the third exon encodes the third and fourth Greek key motifs [27,28]. Each Greek motif consists of a torqued β-pleated sheet resembling the classical Greek pottery [29].

An increase in the number of mutations in the CRYG genes in association with human congenital cataracts has been reported. To date, mutations of crystalline genes represent about half of all mutations reported in familial cataracts. Nuclear cataract consists of opacities in the embryonic and fetal nuclei of the lens. Some patients harbor a dense central opacity surrounded by fine dots, others present fine dot opacities involving embryonic and fetal nuclei with opacification of Y sutures. Our family showed clinical heterogeneity, patient II-1 presented a dense central opacity surrounded by discrete opacity of the cortex (Figure 2A) and high degree of myopia, while patient II-3 had slight asymmetric affection of both eyes (Figure 2B). However, the major morphologic difference was the absence of cortical changes in the female sibling. This phenotypic heterogeneity could be attributed to epigenetic or/and environmental factors.

Several mutations in the CRYGD gene with a wide phenotypic variability have been reported [23,30-37] (Table 2). Molecular analysis of the CRYGA-D cluster of the affected members of the family showed a novel heterozygous missense mutation c.320A>C within exon 3 of CRYGD that encodes the third and the fourth Greek key motifs. This transversion mutation resulted in the substitution of glutamic acid 107 by an alanine. Alanine is a small (MW 89.09) neutral, hydrophobic amino acid, while glutamic acid is larger (MW 147.13) with a negatively charged carboxylate group. The X-ray structure of the wild type γD-crystallin protein [38] has been solved at high resolution (Protein DataBank IDF: 1hk0). Residue E107

<table>
<thead>
<tr>
<th>Codon</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Phenotype</th>
<th>Reference</th>
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<tr>
<td>14</td>
<td>cCGC-TGC</td>
<td>Arg-Cys</td>
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<tr>
<td>23</td>
<td>cCCC-ACC</td>
<td>Pro-Thr</td>
<td>Lamellar cataract, Cerulean cataract, Fasciculiform cataract, Coralliform cataract, Nuclear cataract</td>
<td>[32-36]</td>
</tr>
<tr>
<td>36</td>
<td>gCGC-AGC</td>
<td>Arg-Ser</td>
<td>Cataract with protein crystallization, Congenital nuclear cataract</td>
<td>[31,37]</td>
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<tr>
<td>58</td>
<td>CGC-CAC</td>
<td>Arg-His</td>
<td>Aculeiform cataract</td>
<td>[23]</td>
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<tr>
<td>107</td>
<td>GAG-GCG</td>
<td>Glu-Ala</td>
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<td>This paper</td>
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<tr>
<td>156stop</td>
<td>TGG-TAG</td>
<td>Trp-Term</td>
<td>Central nuclear cataract</td>
<td>[32]</td>
</tr>
</tbody>
</table>

Mutations in CRYGD causing cataract.
is on the surface of the molecule interacting with water. Several other cataract mutations that change the solvation property of an amino acid residue predicted to be on the surface of the γD-crystallin protein molecule have been shown to drastically lower the protein solubility [39] (Table 2). In this study we suggest that c.320A>C mutation in the CRYGD gene also results in a reduction in the protein solubility, which in turn causes this type of nuclear cataract.

The most likely explanation for the unusual pedigree of our family with two affected members with nuclear cataract and normal parents is germline mosaicism. In an attempt to find evidence of mosaicism, the CRYGD locus was haplotyped. Haplotyping (microsatellite marker D2S365) showed that the mother’s haplotype co-segregated with the disease. This finding suggests that the mother presented the germinal mosaicism, nevertheless, further confirmatory tests for mosaicism is necessary. The chance that a disorder due to a new dominant mutation which occurs more than once in a sibship is very low (considering a median gene mutation rate of approximately 1x10⁻⁶ for the event). Two identical events in the same gene in the same family are very unlikely. Diseases with different inherited patterns and somatic and/or germlinal mosaicism have been reported [40-42]. In most cases, genetic mosaicism is due to different aberrant mechanisms as unequal homologous crossover, unequal sister or non-sister chromatid exchanges, excision of intrachromatid loops, or inadequate incorporation of nucleotides in the replication process. Occurrences of mosaicism seems to be more common than previously thought and this possibility must be considered for genetic studies, particularly for prenatal diagnosis or genetic counseling.

In conclusion we report a novel missense mutation (E107A) in the CRYGD gene associated with nuclear cataract and show evidence that the origin of the mutation was in the mother of the patients.

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REFERENCES
13. Litt M, Carrero-Valenzuela R, LaMorticella DM, Schultz DW, Mitchell TN, Kramer P, Maumenee IH. Autosomal dominant cataract results in a reduction in the protein solubility, which in turn causes this type of nuclear cataract. In conclusion we report a novel missense mutation (E107A) in the CRYGD gene associated with nuclear cataract and show evidence that the origin of the mutation was in the mother of the patients.

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